Influenza virions are formed at special ''budding sites'' at the host cell's plasma membrane but the exact molecular mechanisms and spatio-temporal order of virion assembly/egress are poorly characterized, while constituting attractive targets for anti-viral drugs. Here, we optically ''dissect'' these processes to obtain a comprehensive assembly model at nanoscale level. We analyzed the spatio-temporal distribution of viral components, including the ''spike proteins'' hemagglutinin (HA) and neuraminidase (NA), the capsid-forming matrix protein M1, and the nucleoprotein NP, the core of the viral ribonucleoparticles. Using immunofluorescence-based STORM, we detect the various viral constituents at the membrane with high molecular specificity and sensitivity at a resolution of ~ 30nm. Viral protein clusters grow in a time-dependent fashion, with length scales of a few nanometers at early times post infection to dense clusters of several hundred micrometers after hours. Using multicolor-STORM, we can analyze the molecular composition of these clusters at different times, and find different size/shape characteristics for the different proteins. Furthermore, 3D-STORM imaging allows us to detect and to distinguish individual, micrometer-long HA-coated filaments emanating from the plasma membrane, and measure their cross-sections to ~100 - 150nm. These subdiffraction-limit data serve us as invaluable basis to characterize of the assembly process in the cell context at nanoscopic detail.

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GP10 of Bacteriophage Phi29 Exhibits ATPase Activity

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Previously thought to function only as a passive portal for the phi29 DNA packaging motor, we demonstrate that the connector protein gene product 10 (gp10) exhibits significant ATPase activity. Bioluminescence assays have shown a concentration dependent decrease in available ATP after the addition of gp10. Also, enzyme-linked inorganic phosphate assays (ELIPA) have confirmed a time-dependent increase of phosphate in the presence of gp10 and ATP. Alone, gp10 acts as a slow ATPase with optimal hydrolysis of ATP occurring above room temperature at conditions native to the phi29 DNA motor. Overall increase of ATP hydrolysis in the system has been observed upon the addition of other phi29 motor components to gp10. Compared to gp16, a known ATPase, gp10 consumes ATP at a notably slower rate. Moreover, pRNA has shown no measurable ATPase activity. Combined, the phi29 motor assembly (i.e. gp10, gp16, and pRNA) has exhibited greater rates of ATP hydrolysis than any of these components independently.

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The mechanical properties of the icosahedral shell of Southern Bean Mosaic Virus - A molecular dynamics study

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Viruses are assemblies of multi-proteins forming the shell and the genetic material that is protected inside. Until know the process of assembly and viral infection remains unclear and the investigation of mechanical properties plays an important role here, as well as in understanding (1) How is the DNA/RNA packed inside and how can a protein shell withstand internal pressures of more than 60 atm? (2) How are the elastic properties distributed on the viral surface and how do they change before infection can take place? To address these questions, we performed force-probe molecular dynamics simulations on the complete shell of Southern Bean Mosaic Virus, a typical representative of RNA viruses with T=3 symmetry. The whole simulation system, including 1,000,000 water molecules, comprises more than 4,500,000 atoms, to our best knowledge one of the largest biomolecular simulation systems in the world. To facilitate direct comparison to recent atomic force microscopy measurements, a Lennard-Jones sphere served as a model of an AFM tip and was pushed with a constant velocity towards 19 different grid points on the capsid surface. An inhomogeneous distribution of elastic constants and rupture forces was found. The strongest elastic response was seen in the center of the pentamers at the five-fold symmetry axis. Furthermore, we investigated the effect of Ca^{2+} removal on the elasticity because this removal is supposed to by the first step in cell infection. We see a marked weakening along the five-fold symmetry axes which suggests that the pentamers serve as a possible gate for RNA release.

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Single Particle Force Spectroscopy Reveals Virus DNA Storage Strategies And Structural Properties Of Capsids And Virions

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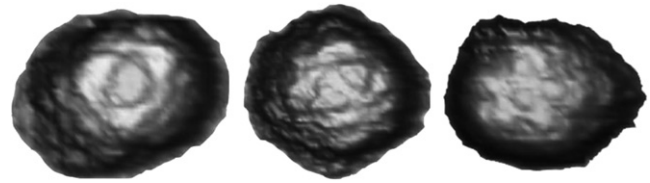
By using Atomic Force Microscopy in buffer conditions we performed nanoindentation experiments on several kinds of viruses. In the spherical virus Minute Virus of Mice we discovered that the DNA molecule exerts an architectural role by increasing the stiffness of the virus on symmetries axis 3 and 2. This mechanical reinforcement is a consequence of the interaction between crystallographically visible, short DNA patches and the inner capsid wall, and apparently does not build internal pressure inside the virus.

However, preliminary experiments performed on T7 bacteriophage capsids and virions reveal that DNA storage produces an internal pressure of around 30 atmospheres, comparable to that found in other bacteriophages such us phi-29. We have also visualized for the first time the topography of in situ reversible buckling events in a capsid (T7) which shows the behaviour of the intercapsomers bonds under stress.

We have studied the existence of built in stress in the equatorial zone of phi-29 which can not be account by using continuum mechanics models.



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Protein Unfolding Revealed by Factor Analysis of Raman Spectra: Application to HK97 Virus Assembly

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Double-stranded DNA virus assembly involves precisely coordinated interactions of hundreds of identical protein subunits to form a precursor shell that is subsequently transformed into the mature shell of the native virion at the time of DNA packaging. While precursor shell assembly typically requires a chaperone-like protein (scaffolding) to ensure formation of the properly dimensioned particle, no scaffolding is required to assemble the HK97 virus. Instead, a multi-domain subunit (gp5) self-assembles to yield the required precursor shell architecture (Prohead I), and the gp5 subunits of this shell are subsequently cleaved between residues 103 and 104 by a viral protease to eliminate the N-terminal domain (Delta-domain). The resulting metastable shell (Prohead II) is competent to package DNA and eventually mature to the native virus architecture (Head). Although the structures and stabilities of Prohead I, Prohead II and mature Head of HK97 have been investigated, little is known about the structure and stability of the Delta-domain and its role in the Prohead I to Prohead II transformation. We have addressed this problem using a novel factor analysis approach; namely, singular value decomposition (SVD) of temperature-dependent Raman spectra. The structure and stability of two distinct forms (states) of the Delta-domain have been investigated: (i) a recombinantly expressed 111-residue Delta-domain *in vitro*, which is free of interactions with other domains of the Prohead I subunit, and (ii) the *in situ* Delta-domain of the gp5 subunit of the native Prohead I assembly. Raman spectra were analyzed over the interval 10-90°C. SVD analysis has provided the key thermodynamic parameters of the complete Gibbs-Helmholtz equation. Simultaneously, these data have allowed evaluation of secondary and tertiary structural changes accompanying Delta-domain unfolding in the two Delta-domain states. [Supported by NIH grant GM50776.]

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Internal Capsid-Pressure Dependence of Viral Infection by Phage Lambda

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Ejection of the genome from the virus, phage λ, is the initial step in the infection of its host bacterium. In vitro, the ejection depends sensitively on internal pressure within the virus capsid; however, the effect of internal pressure on infection of bacteria is unknown. Here, we use microfluidics to monitor individual cells and determine the temporal distribution of lysis due to infection as the capsid pressure is varied. The lysis probability decreases markedly with decreased capsid pressure. Interestingly, the average lysis times remain the same, but the distribution is broadened, as the pressure is lowered.